helices are only straight because the voltage sensor, via the S4-S5 linker, is held in the down position by the transmembrane voltage. This has important consequences for efforts to try and crystallize a voltage-gated potassium channel in the closed state.

Platform AT: Biotechnology & Bioengineering

1983-Plat
Development of an Optogenetic Sensor of Membrane Potential
Joel M. Kralj, Daniel R. Hochbaum, Adam D. Douglass, Adam E. Cohen.
Harvard University, Cambridge, MA, USA.
Living organisms use voltage to transmit information over distances ranging from nanometers to meters. Traditional probes of voltage rely on physical electrodes which are invasive and can be used only in limited numbers. Here we present a voltage-sensitive fluorescent protein based on green-absorbing proteorhodopsin. The Proteorhodopsin Optical Proton Sensor (PROPS) has a far red excitation, a near infrared emission, is highly photostable, and is exquisitely sensitive to membrane potential. This protein has no homology to GFP or to any existing indicator of membrane potential. Using PROPS we have recorded for the first time electrical activity in live bacteria and saw 5-fold changes in fluorescence. We have also put it into eukaryotic cells in order to record electrical activity from neurons. PROPS represents a new optogenetic tool in the quest for an all optical neural interface.

1984-Plat
Expanding the Scope of Single Molecule FRET with DNA Origami
Carlos E. Castro, Hendrik Dietz.
Technische Universität München, München, Germany.
Typical dimensions of cellular components are on the order of a few nanometers and their diverse cellular functions often involve conformational changes that require movements ranging from fractions of a nanometer up to tens of nanometers. The current state of the art tool for studying conformational dynamics is single molecule Fluorescence Resonance Energy Transfer (FRET), whereby energy transfer between two fluorescent dyes is correlated to their spatial separation; however, distance predictions made by FRET theory are only accurate in the range of ~ 3-7 nm, and the working range is limited to below 10 nm. Furthermore, quantitative distance predictions require case-specific calibrations.
Here we present a nanoscale device constructed by DNA origami that improves the quantitative accuracy of FRET distance predictions and expands its potential working range. The device integrates 3d structures built from self-assembled DNA, attachment sites for molecules of interest, sites for surface immobilization, and fluorescent markers for the direct visualization of biomolecular dimensions and dynamics by FRET. The device takes advantage of a distance calibration that can be generally applied to any molecule of interest. We have demonstrated that the device can easily achieve 1 nm distance resolution. For the purpose of proof-of-concept studies we specifically integrated a piece of double stranded DNA (dsDNA) containing a recognition sequence for Catabolite Activator Protein (CAP), which is know to bend dsDNA upon binding, between the arms of this device. The bending angle will be evaluated with single particle electron microscopy (EM), and FRET microscopy will be employed in solution to resolve real-time kinetics and deformations of CAP-DNA binding.

1985-Plat
***In vitro* and *In silico* Optogenetic Control of Differentiated Human Pluripotent Stem Cells**
Oscar Abilez, Rohit Prakash, Jonathan Wong, Ellen Kuhl, Karl Deisseroth, Christopher Zarins.
Stanford University, Stanford, CA, USA.
Purpose: Despite the use of a variety of differentiation protocols, no method exists to prospectively generate specific phenotypes of human embryonic stem cell (hESC)-derived cardiomyocytes (CM) such as pacemaker cells. We introduced light-activated channelrhodopsin-2 (ChR2) into hESC, and by using *in vitro* and *in silico* approaches, we were able to optogenetically synchronize hESC-CM, both experimentally and computationally.
Methods: We experimentally introduced ChR2 coupled to yellow fluorescent protein (YFP) into undifferentiatedhESC via a lentiviral vector and tested for expression via PCR, flow cytometry (FC), and immunocytochemistry (ICC). $hESC^{ChR2+}$ were sorted, expanded, and tested for pluripotency. Via directed differentiation, wildtype hESC-CM and ChR2-CM were produced and subjected to both electrical and optical stimulation. Electrical, biochemical, and mechanical signals were then assessed by patch clamping, multielectrode arrays (MEAs), and video microscopy. To complement our *in vitro* approach with *in silico* analyses, we introduced ChR2 into an ionic cardiac cell model.
Results: ChR2 was stably transduced into undifferentiated hESC and the resulting $hESC^{ChR2+}$ pluripotent line could be differentiated into CM, all confirmed by PCR, FC, ICC, and electrophysiological methods. Both WT-CM and ChR2-CM responded to traditional electrical stimulation and produced similar calcium and contractility features but only ChR2-CM could be synchronized by optical stimulation. In addition, by calibrating our ionic cell model with single cell action potential (AP) readings, we were able to virtually probe the impact of photostimulation stimulus amplitude, pulse width, and frequency on overall AP characteristics.
Conclusions: Here we show for the first time that ChR2 can enable *in vitro* and *in silico* optical control of hESC-CM. The long-term application of optical stimulation could potentially lead to specific synchronous phenotypes of hESC-CM. This, in turn, would contribute significantly towards creating effective therapies for cardiovascular disease.

1986-Plat
Fluorogenic Pyrosequencing in Microreactors
William J. Greenleaf, Peter A. Sims, Haifeng Duan, Xiaoliang Sunney Xie.
Harvard University, Cambridge, MA, USA.
High-throughput sequencing techniques are revolutionizing biology and promise to have a significant impact on the future of medicine. We have developed a scalable, parallel, low-cost method for nucleic acid sequencing that combines resealable microreactors and terminal-phosphate labeled fluorogenic nucleotides. To accomplish this, we have fabricated arrays of microreactors in polydimethylsiloxane (PDMS) that can be reversibly sealed, and have developed techniques for immobilizing different populations of identical DNA fragments specifically and efficiently within these microreactors. We have synthesized a series of terminal-phosphate labeled fluorogenic substrates for DNA polymerase which, upon their incorporation into a primer strand of DNA and subsequent digestion by a phosphatase, generate fluorescent dye molecules that are effectively trapped in the microreactors. By sequentially interrogating the microreactor array with solutions containing different fluorogenic nucleotide substrates, and recording which microreactors generate fluorescence, we can sequence the immobilized DNA. This "fluorogenic pyrosequencing" method promises significant advantages over existing sequencing technologies, and has the potential to be elegantly interfaced with the wide variety of PDMS-based microfluidic devices suitable for the preparation and amplification of DNA or RNA for sequencing, opening the possibility of one-chip, sample-to-sequence capabilities.

1987-Plat
Mapping Virus-Host Protein Interactions using the Ping Microfluidics Platform
Doron Gerber[1], Shirit Einav[2], Stephen R. Quake[2].
[1]Bar Ilan University, Ramat Gan, Israel, [2]Stanford University, Stanford, CA, USA.
During their life cycle viruses hijack host networks for their own needs. Discovering the basic biochemical interactions between viral proteins and host proteins is a crucial step towards understanding the viral life cycle. Due to the size of the host proteome high-throughput methods are needed to screen for such viral-host interactions. Despite significant efforts, conventional tools such as Yeast Two Hybrid largely failed. The failure is more pronounced when it comes to membrane associated proteins. We have designed PING, a sensitive high-throughput microfluidic assay that allows the screen of viral-host protein interactions as well as screen for inhibitors of such interactions. In a proof of principle experiment we screened the two HDV proteins and HCV NS5A against a hundred human proteins. We identified 7, 4 and 6 novel partners for sHDAg, LHDAg and NS5A, respectively. The average sensitivity of a single experiment (known partners identified out of all known partners included) was 62%, similar to bacterial interaction data previously measured with PING. The cumulative sensitivity in 4 experiments was 100%. These results pave the way for a whole proteome screen, which will allow us the horizontal overview we need into the biochemical network of viruses inside their host.